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ORIGINAL PAPER

# Effect of arbuscular mycorrhizal fungi on growth of *Gmelina arborea* in arsenic-contaminated soil

A. Barua • S.D. Gupta • M.A.U. Mridha • M.K. Bhuiyan

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**Abstract:** Arsenic (As) in the soils of South-Eastern Bangladesh is not only a threat for the health of millions of people but also a problem for plant growth due to its higher concentration in soil. *Gmelina arborea* Linn. is a promising fast growing tree species in Bangladesh which has also a potential to be planted in arsenic contaminated areas. This study assessed the role of arbuscular mycorrhizal (AM) fungi on the growth of *G. arborea* in arsenic amended soils at nursery stage. Before sowing seeds, soils were treated with four different concentrations (10 mg·kg$^{-1}$, 25 mg·kg$^{-1}$, 50 mg·kg$^{-1}$, and 100 mg·kg$^{-1}$) of Arsenic. Growth parameters (length of shoot and root, collar diameter, fresh and dry weight of shoot and root) of the plant, and mycorrhizal root colonization and spore population in the rhizosphere soil of *G. arborea* were recorded. Mycorrhizal seedlings showed better growth than non-mycorrhizal seedlings. Mycorrhizal seedlings planted in soil with 10-mg·kg$^{-1}$ arsenic showed best performance in terms of growth, biomass and mycorrhizal colonization, compared to other treatments with higher concentration of arsenic. With increasing arsenic concentration, growth of seedlings, mycorrhizal infection rate and spore population, all decreased significantly ($p$<0.05). The mycorrhizal seedlings had as much as 40% higher increment in total growth and 2.4 times higher increment in biomass compared to non-mycorrhizal seedlings. The study clearly indicated that mycorrhizal inoculation could reduce the harmful effects of arsenic on the initial growth of *G. arborea* Linn. in degraded soil at nursery stage.

**Keywords**: arsenic; arbuscular mycorrhizal fungi; *Gmelina arborea* Linn.; bioremediation; plant growth

## Introduction

Arsenic (As) contamination is posing a serious threat in several regions of Asia (Heikens 2006). Arsenic, its fate and transport in the environment have become matters of great concern in Bangladesh, India and several other countries (Ali et al. 2003). According to the WHO report, Bangladesh falls in the severe As-contaminated zone in South Asia and the concentration level was found to be 13–21 mg·kg$^{-1}$ in the soil of Bangladesh (Islam et al. 2000). Distribution of arsenic ranged from 10 to 15 mg·kg$^{-1}$ at above 15-cm soil depth. Very few cases were found with the levels more than 20 mg·kg$^{-1}$ (Huq et al. 2003; Duxbury and Zavala 2005; Shah et al. 2004). The problem is severe mostly in southern region, some parts in north-east and very sporadically in north-western region of Bangladesh (British Geological Survey 1998). Over exploitation of ground water is the most agreed cause of arsenic toxicity in soils of Bangladesh as this activity lowered the ground water table and increased arsenic concentration in the surface ground water level (Das et al. 1996). Various reports indicated that arsenic concentration is increasing in soils of Bangladesh because of arsenic input via irrigation water, and it is now appeared as a major environmental concern (Chowdhury et al. 2000; Mukherjee and Bhattacharya 2001; Alam et al. 2002; Chakraborti et al. 2002).

Arsenic is found everywhere in nature, in most soils and rocks. The presence of arsenic in soil seems to be toxic to plants and may be accumulated in plant parts and thereby enters into animal as well as into human through the food chain. The concentration of arsenic in uncontaminated soil ranges from 0.2 to 40 mg·kg$^{-1}$ which is not likely to cause any phytotoxicity (Fowler et al.

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A. Barua
Institute of Forestry and Environmental Sciences, University of Chittagong, Chittagong-4331, Bangladesh. Email: amit_ctg2008@yahoo.com

S. D. Gupta (✉)
School of Environment, Natural Resources and Geography, Bangor University, Gwynedd LL57 2DG, United Kingdom.
E-mail: sdgupta07@gmail.com

M.A.U. Mridha
Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh.

M.K. Bhuiyan
Institute of Forestry and Environmental Sciences, University of Chittagong, Chittagong-4331, Bangladesh

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2007). The total amount of arsenic in soil and its chemical forms have an important influence on plant growth as well as animal and human health (Yan-Chu 1994). Inorganic arsenic is much more hazardous than organic form (Adriano 2001). Bioaccumulation of arsenic represents a high human health risk, which is directly related with cancer, arteriosclerosis, chronic liver disease and other health problems. Plant root contains higher proportion of arsenic than any other plant parts. The edible parts of plant rarely contain hazardous level of arsenic. Subjection of arsenic toxicity to human and animal health can result from ingestion of surface residues of arsenic in plant. The amount of arsenic in soil is directly correlated with the amount of arsenic in the whole plant (Walsh et al. 1977). Plants show variable tolerance limit to arsenic toxicity. Low level of arsenic is often found to stimulate plant growth although it is not a nutrient element for plant. Plants suffer growth suppression at higher level of arsenic contamination in soil. Inorganic form of arsenic (e.g. arsenite, arsenate, etc.) is highly toxic for plant membrane as it reacts with cell protein, disrupts root functions, and inhibits nutrient uptake process of leaves. The toxicity can also stop seed germination and cause death of plant cells (Carbonell et al. 1998).

Among several factors determining arsenic uptake and toxicity in plants, one of the most important factors is the form of arsenic. The two most important forms of arsenic, arsenic-V and arsenic-III, are taken up by plants in completely different mechanisms. Arsenic uptake, accumulation and toxicity vary within and between plant species. Permissible level of arsenic in agricultural soil of Bangladesh is 20 $mg \cdot kg^{-1}$ and toxicity level starts from 5 $mg \cdot kg^{-1}$ for crops with variability like 20 $mg \cdot kg^{-1}$ for barley and 100 $mg \cdot kg^{-1}$ for rice (Hasan 2009). However, threshold level of tree species for resistance of arsenic toxicity is still poorly known. In general, more arsenic in the soil leads to higher concentrations in plants. It is not yet possible to predict arsenic uptake and/or remediate arsenic toxicity in plants from soil parameters (Heikens 2006). Arbuscular mycorrhizal (AM) fungi are well known for its potential role in the growth of host plants by increasing the nutrient uptake ability and tolerance to adverse conditions (Smith and Read 1997). In contaminated soil, AM fungi act as a barrier of uptaking toxic metals by host plant (Leyval et al. 1997). Out of the different types of the mycorrhiza, the AM fungi are very important in relation to the improvement of plant growth and nutrient status in arsenic-contaminated soils (Ultra et al. 2007).

*Gmelina arborea* Linn. (English name, Beechwood; Local name, Gamar) is a moderate-sized deciduous tree. Due to its ability of growing in very poor soils or in stressed condition, *Gmelina arborea* Linn. has become popular in different plantation programs of agroforestry, community forestry, social forestry, village and farm forestry in different regions of Bangladesh and several other tropical countries (Snelder and Lasco 2008; Nath and Inoe 2008). To fulfill the demand in the plantation programs, many organizations are producing *G. arborea* in the nursery in Bangladesh. The species is widely used for pulpwood, fuelwood, shade, reclamation, afforestation program and timber production (Wingfield and Robison 2004; Mantel et al. 2006). *G. arborea* can be a potential species for plantation in arsenic-affected areas of the country. Although, *G. arborea* is well concerned as a stress resistant tree species, its initial growth performance and symbiotic effects of mycorrhizal inoculation have not yet been studied in arsenic-contaminated soils of Bangladesh. Knowledge of symbiotic performance of *G. arborea* in arsenic-contaminated soil can have substantial impact in establishing a successful plantation program. Most of the researches on determining arsenic-toxicity level and remediation measures in Bangladesh were dealt with agricultural crops (Huq et al. 2006; Hossain 2006). To our knowledge, performance of tree species in arsenic-contaminated soil is still poorly understood. This study can give an idea how tree seedlings response with increasing arsenic level in soil and create an avenue for bioremediation of arsenic toxicity in soil. Thus, the aim of the study was to reveal the influence of AM fungi on the growth of *G. arborea* in arsenic-amended soil at nursery stage and also to investigate AM fungal infectivity in plant root at different levels of arsenic.

## Materials and methods

### Seed collection, treatment and soil preparation

The experiment was carried out from September to December, 2006 in the nursery of the Institute of Forestry and Environmental Sciences, University of Chittagong, Bangladesh (91°50'E latitude and 22°30'N longitude). Seeds of *G. arborea* were collected during the fruiting season (March-June) from Chittagong University and they were sown after soaking in water for 24 h. Soils were collected from hilly sites of Chittagong University campus. The collected soils were then sieved (2 mm mesh) to remove the non-soil materials and used for filling poly bag for seed sowing. Each poly bag (15 cm× 23 cm) was filled with approximately 2-kg sieved soil leaving upper 5 cm of poly bag vacant to facilitate watering.

### Preparation of arsenic solution and Mycorrhizal inoculum

Exactly arsenic trioxide of 1.32 g was dissolved in 1-M NaOH, made acidic by adding dilute (0.1M) HCl and total volume was made to 1000 mL by adding distilled water. The standard solution was contained arsenic of 10 $\mu g \cdot mL^{-1}$. From stock solution of arsenic, different concentrations of arsenic solution were prepared according to experimental design and mixed up with soil up to the saturation point.

The inoculum was prepared from the roots and rhizosphere soils of *Leucas aspera.* The roots of *L. aspera* with rhizosphere soils were collected from Chittagong University campus. Collected roots were surfaced sterilized by dipping into 70% ethanol for 2 min and then chopped into 1-cm segments. The infectivity of the roots was tested and 100% colonization was observed with an average of 125 spores per 100 g of rhizosphere soil. About 15 g of root inoculum was incorporated in the upper part of the each experimental pot and a 1-cm soil layer was added on the inocu-

lum layer.

Experimental design

The experiment was arranged in Randomized Complete Block Design (RCBD). Total number of treatments was 10 and total replications were 180 (18 seedlings per treatment). Two seeds were sown in each pot at 1-cm soil depth. The treatment was sustained with only one vigorous seedling and the other seedlings were removed from the pot. The ten (10) treatments were as followed:

$T_1$- Fresh soil (control),
$T_2$- Soil + Mycorrhiza,
$T_3$- Soil + Arsenic (As) of 10 mg·kg$^{-1}$,
$T_4$- Soil + As of 10 mg·kg$^{-1}$ + Mycorrhiza,
$T_5$- Soil + As of 25 mg·kg$^{-1}$,
$T_6$- Soil + As of 25 mg·kg$^{-1}$ + Mycorrhiza,
$T_7$- Soil + As of 50 mg·kg$^{-1}$,
$T_8$- Soil + As of 50 mg·kg$^{-1}$ + Mycorrhiza,
$T_9$- Soil + As of 100 mg·kg$^{-1}$,
$T_{10}$- Soil + As of 100 mg·kg$^{-1}$+ Mycorrhiza.

Seedling harvest and estimation of AM fungal colonization and spore population

Seedlings were harvested after 90 days. Different growth parameters like shoot height and root length, collar diameter, leaf number, dry weight of root and shoot were measured. The colonization percentage of AM fungi in the plant root system and spore population of rhizosphere soils were assessed. Staining of root segments was done following the method of Phillip and Hayman (1970). Roots were cut into 1-cm segments and 100 segments were randomly selected for staining. Root segments were first heated in 10% Potassium Hydroxide (KOH) solution for 90 min at a temperature of 90 ºC to remove cytoplasm and later stained with 0.05% aniline blue prepared in a solution of lactoglycerol. Stained root segments were examined under a microscope for the evaluation of intensity of AM fungal colonization. The colonization intensity is an estimation of the amount of root cortex colonized, determined as the percentage of root length occupied by fungal hyphae, vesicles, and arbuscules. These parameters were calculated as described by Trouvelot et al. (1986).

Arbuscular mycorrhizal (AM) fungal spore enumeration was done according to sieving and decanting method (Gerdemann and Nicolson 1963). Soil was dispersed in 1-liter deionized water and the suspension was left to be undisturbed for 5 min to allow the heavier particles to settle down. Then the suspension was decanted through 400-µm, 240-µm and 60-µm sieves gradually to extract the spores. Larger spores were separated from the supernatants of the sieves of 400 µm and 240 µm by soft forceps and relatively smaller spores were collected in a wash glass from the 60-µm sieves with water. The suspension of water and spores were filtered by the Whatman 42 filter paper. Total numbers of spores were counted per 100 g of dry soil basis.

Mycorrhizal dependency (MD)

Mycorrhizal dependency (MD) values of *G. arborea* Linn. were calculated by expressing the difference between the total dry biomass of the mycorrhizal and non-mycorrhizal seedlings as the percentage of the dry biomass of mycorrhizal seedlings (Plenchette et al. 1983).

Statistical analysis

The data were statistically analyzed using Analysis of Variance (ANOVA) and the means were separated by Duncan's Multiple Range Test ($p < 0.05$) using Statistical Package for Social Sciences (SPSS v 16.0) and MS Excel (MS Office 2007).

## Results

Physical growth parameter of seedlings planted in soil with different concentrations of arsenic is presented in Table 1 and Table 2. Amongst all the treatments with arsenic, best performances were found in seedlings planted in treatment $T_4$. With increasing arsenic level in soil, growth parameters decreased gradually.

**Table 1. Shoot height, root length, collar diameter and leaf number (mean±SE) of *G. arborea* grown in soil treated with different concentrations of arsenic**

| Treatments | Length (cm) | | | Collar dia.(cm) | No. of leaf |
|---|---|---|---|---|---|
| | Shoot | Root | Total | | |
| $T_1$ | 20.1±1.25c* | 17.7±0.53c | 37.8±1.02c | 1.1±0.16cd | 6±1.05cd |
| $T_2$ | 25±0.87a | 20.1±0.32a | 45.1±0.98a | 1.5±0.27a | 7±1.13a |
| $T_3$ | 16.1±0.88g | 13.1±0.33g | 29.2±0.81g | 0.6±0.13g | 6±0.87bcd |
| $T_4$ | 22.2±0.72b | 18.4±0.34b | 40.6±0.85b | 1.2±0.26bc | 7±0.97a |
| $T_5$ | 15±0.56h | 11.7±0.50h | 26.7±0.89h | 0.4±0.12hi | 5±1.05de |
| $T_6$ | 19.3±0.87d | 16.2±0.41d | 35.5±1.14d | 1±0.16de | 7±1.37ab |
| $T_7$ | 13.4±0.75i | 11.4±0.34i | 24.8±0.84i | 0.3±0.12hi | 4±1.07ef |
| $T_8$ | 18.2±0.59e | 15.6±0.46e | 33.8±0.75e | 0.9±0.15ef | 6±1.39abc |
| $T_9$ | 11.0±0.73j | 10.3±0.51j | 21.3±0.97j | 0.1±0.02j | 3±0.99f |
| $T_{10}$ | 15.4±0.74f | 14.4±0.45f | 29.8±0.91f | 0.8±0.16ef | 5±1.10de |

**Notes**: *Means followed by the same letter (s) in the same column do not vary significantly at $p<0.05$, according to Duncan's Multiple Range Test (DMRT).

Shoot and root length, collar diameter and leaf number

Shoot and root length, and collar diameter of *G. arborea* seedlings varied significantly ($p<0.05$). No significant difference was found in case of leaf number except between treatment $T_8$ and $T_{10}$. Mycorrhizal seedlings showed comparatively higher growth than non-mycorrhizal seedlings. The highest shoot growth (22 cm; 2.4 mm·d$^{-1}$), root growth (18.4 cm; 2 mm·d$^{-1}$), collar diameter (1.2 cm; 0.13 mm·d$^{-1}$) and leaf number (7) were observed in treatment $T_4$ amongst the seedlings planted in arsenic amended soil. Lowest growth performance was observed in seedlings

planted in soil treated with 100-mg·kg$^{-1}$ arsenic ($T_9$). Shoot height, root length, collar diameter and leaf number of *G. arborea* seedling in treatment $T_9$ were 11 cm, 10.3 cm, 0.1 cm and 3, respectively. Seedlings grown in control treatment ($T_2$) without arsenic showed best growth performance among all the treatments. Mean shoot height, root length, collar diameter and leaf number for this treatment were 25 cm, 20.1 cm, 1.5 cm and 7, respectively (Table 1).

**Table 2. Fresh and dry weight of *G. arborea* shoot and root (mean ± SE) grown in soil treated with different concentrations of arsenic**

| Treatments | Fresh weight (g) | | | Dry weight (g) | | |
|---|---|---|---|---|---|---|
| | Shoot | Root | Total | Shoot | Root | Total |
| $T_1$ | 1.6±0.47b* | 1.06±0.02d | 2.67±0.47c | 0.51±0.05c | 0.58±0.04c | 1.09±0.34c |
| $T_2$ | 2.7±0.27a | 1.81±0.20a | 4.51±0.33a | 0.78±0.05a | 0.80±0.04a | 1.58±0.16a |
| $T_3$ | 0.78±0.03de | 0.43±0.04ef | 1.22±0.06d | 0.25±0.03fg | 0.34±0.04e | 0.59±0.03g |
| $T_4$ | 1.74±0.21b | 1.66±0.22b | 3.40±0.13b | 0.69±0.05b | 0.66±0.04b | 1.35±0.41b |
| $T_5$ | 0.68±0.04ef | 0.36±0.035f | 1.04±0.06e | 0.20±0.03f | 0.30±0.03f | 0.50±0.05h |
| $T_6$ | 1.31±0.04c | 1.23±0.16b | 2.54±0.19c | 0.41±0.04d | 0.60±0.05d | 1.01±0.05d |
| $T_7$ | 0.51±0.04f | 0.18±0.024g | 0.69±0.05f | 0.12±0.02g | 0.20±0.03f | 0.32±0.03i |
| $T_8$ | 0.88±0.03d | 0.47±0.033e | 1.35±0.04d | 0.30±0.03e | 0.41±0.04e | 0.71±0.04e |
| $T_9$ | 0.11±0.02g | 0.07±0.018h | 0.18±0.03g | 0.10±0.02h | 0.18±0.02g | 0.28±0.30j |
| $T_{10}$ | 0.52±0.03f | 0.35±0.024f | 0.87±0.03e | 0.28±0.06f | 0.39±0.03f | 0.67±0.05f |

**Notes**: *Means followed by the same letter(s) in the same column do not vary significantly at $p<0.05$, according to Duncan's Multiple Range Test (DMRT).

Fresh and dry weight of shoot and root

Both fresh and dry weights of shoot and root differed significantly ($p < 0.05$) among mycorrhizal and non-mycorrhizal plants. Seedlings in treatment $T_4$ had the maximum fresh and dry weights of shoot among the arsenic-mycorrhizal treatments. Maximum fresh weight of shoot was 1.74 g (0.19 mg·d$^{-1}$) and dry weight was 0.69 g (0.07 mg·d$^{-1}$) (Table 2). Similarly, maximum root weight was also found in treatment $T_4$. Maximum Fresh and dry weight of root in $T_4$ was 1.66 g (0.18 mg·d$^{-1}$) and 0.66 g (0.07 mg·d$^{-1}$), respectively. In all cases, the lowest weight of root and shoot was observed in treatment $T_9$. The highest total dry biomass of *G. arborea* seedling was recorded in treatment $T_4$ (10 mg·kg$^{-1}$ arsenic) followed by $T_6$ (25 mg·kg$^{-1}$ arsenic), $T_8$ (50 mg·kg$^{-1}$ arsenic) and $T_{10}$ (100 mg·kg$^{-1}$ arsenic) amongst the mycorrhizal seedlings. Among all the treatments (both mycorrhizal and arsenic-mycorrhizal), treatment $T_2$ performed best with maximum shoot (fresh weight of 2.7g; dry weight of 0.51 g) and root weight (fresh weight of 1.06 g; dry weight of 0.58 g), (Table 2).

Mycorrhizal colonization

Root colonization of arbuscular mycorrhizal (AM) fungi differed significantly ($p < 0.05$) among mycorrhizal seedlings (Table 3). Mycorrhizal colonization of mycelium (21%), vesicle (19%) and arbuscule (10%) was found to be highest in the treatment $T_4$ (10 mg·kg$^{-1}$ of arsenic with mycorrhiza) amongst the arsenic-mycorrhizal treatments, which successively decreased with increasing arsenic concentration. Lowest root infectivity was found in treatment $T_{10}$ which was mycorrhiza inoculated soil with maximum concentration (100 mg·kg$^{-1}$) of arsenic used in this experiment.

**Table 3. Data on root colonization, mycelium growth and spore population in rhizosphere soil (mean ± SE) of *G. arborea* grown in soil treated with different concentrations of arsenic**

| Mycorrhizal treatments | Mycorrhizal colonization | | | |
|---|---|---|---|---|
| | Infection (%) | | | Spore population |
| | Mycelium | Vesicle | Arbuscule | |
| $T_2$ | 43± 1.61a* | 56±1.23a | 22±0.70a | 42±0.74a |
| $T_4$ | 21±0.81b | 19±0.85b | 10±0.70b | 30±0.57b |
| $T_6$ | 17±1.40b | 18±0.90b | 6±0.67c | 22±0.84c |
| $T_8$ | 10±0.58c | 8±0.61c | 3±0.25d | 18±0.80d |
| $T_{10}$ | 7±0.74c | 3±0.21d | 3±0.33d | 11±0.57e |

**Notes**: *Means followed by the same letter (s) in the same column do not vary significantly at $p<0.05$, according to Duncan's Multiple Range Test (DMRT).

Mycorrhizal treatment with no arsenic ($T_2$) showed highest root infection percentage with a value of 43%, 56% and 22% for mycelium, arbuscule and vesicle, respectively. Reduction in root infectivity and rhizosphere spore population of *G. arborea* seedlings in mycorrhizal treatments with increasing As is shown in Fig. 1. In treatment with 25 mg·kg$^{-1}$ of arsenic ($T_6$), the infection percentage in root reduced by 60%, 67% and 72% for mycelium, vesicle and arbuscule, respectively in comparison with treatment $T_2$ which was only treated with mycorrhiza. Treatment $T_8$ (75 mg·kg$^{-1}$ of arsenic) resulted in lower mycelium, vesicle and arbuscular infection percentage by 76%, 85% and 86%, respectively in comparison with treatment $T_2$. Spore populations in the rhizosphere soil also showed significant ($p<0.05$) variation among the treatments and populations increased with decreasing arsenic concentration. Highest spore populations (30) were found in treatment with the lowest arsenic concentration ($T_4$). In treatment $T_{10}$, spore

populations were the lowest of all treatments with a value of 11. With increasing arsenic concentration in soil, spore populations reduced gradually by 29%, 48%, 57% and 74% for treatment $T_4$, $T_6$, $T_8$ and $T_{10}$, respectively in comparison with arsenic free treatment $T_2$ where spore populations were 42 per 100 g of rhizosphere soil.

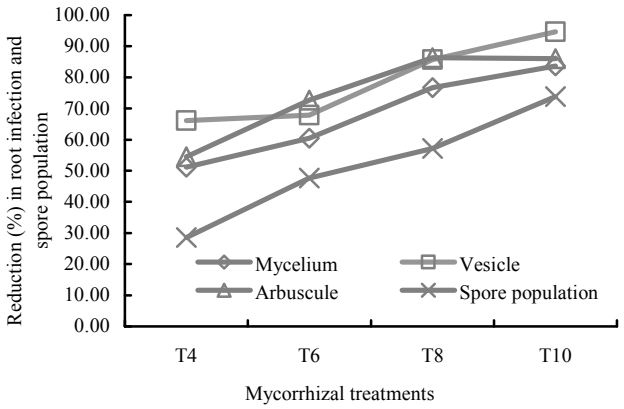


**Fig. 1 Percentage (%) reduction in root infection (mycelium, vesicle and arbuscule) and rhizosphere spore population of *G. arborea* seedlings with higher As concentration in comparison with treatment $T_2$**

Mycelium, vesicle and arbuscule infection and spore population in rhizosphere soil of *G. arborea* Linn seedlings showed significant ($p < 0.05$) positive correlation with total biomass production which signifies the role of mycorrhiza in enhancing seedling growth performance in toxic soil (Fig. 2). Mycorrhizal seedlings attained as much as 40% increment in total growth and 2.4 times (139%) biomass production, compared to non-mycorrhizal seedlings (Fig. 3). Total growth of seedlings planted in soil with arsenic-mycorrhizal treatments ($T_4$, $T_6$, $T_8$ and $T_{10}$) were 39%, 33%, 36% and 40% higher than that of seedlings planted in arsenic treated soil with treatments ($T_3$, $T_5$, $T_7$ and $T_9$), respectively. Likewise, total biomass of arsenic-mycorrhizal seedlings was 129%, 102%, 122% and 139% higher than that of the arsenic-non-mycorrhizal seedlings. Mycorrhizal dependency (MD) of *G. arborea* also differed among treatments with different levels of arsenic (Fig. 4). Highest dependency (58%) was found in treatment $T_{10}$. In arsenic free treatment ($T_2$), MD was the lowest (31%) but started to increase with increasing arsenic concentration in soil. In treatment with 10 mg·kg$^{-1}$ of arsenic ($T_4$), MD increased by 80% in comparison with MD of treatment $T_2$ with an actual value of 56%. In treatment $T_6$, MD increased in a decreasing manner by 68% in comparison with treatment $T_2$. In successive treatments, MD gradually increased with increasing arsenic concentration. MD in treatment $T_8$ increased by 77% and in treatment $T_{10}$, by 87% in comparison with treatment $T_2$. The actual MD of *G. arborea* seedlings in treatment $T_8$ was 55%.

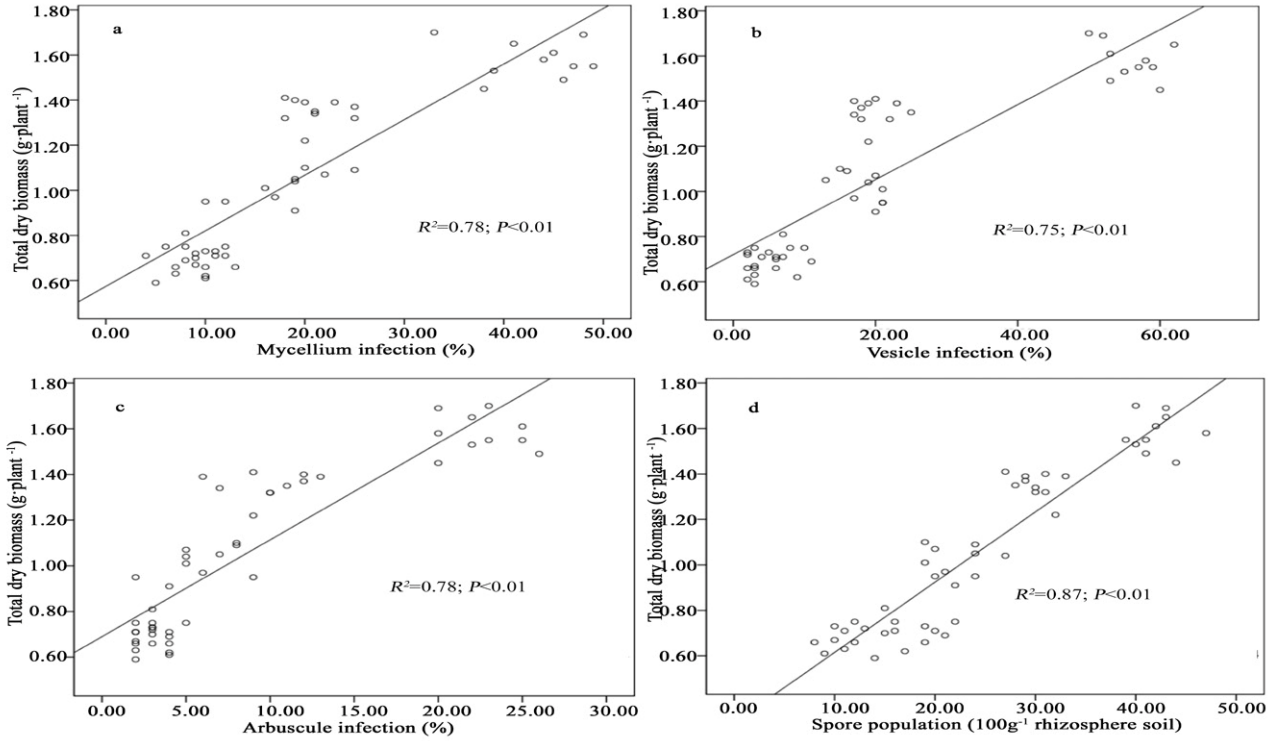


**Fig. 2 Relationship between total biomass and root mycelium (a), vesicle (b), arbuscule (c) infection (%) and AM fungal spore population (d) in rhizosphere soil of *G. arborea* seedlings**

## Discussion and conclusions

Significant effect of arbuscular mycorrhizal (AM) fungi on the growth of *G. arborea* Linn. in arsenic contamination soil was observed in the current experiment. The results indicated that mycorrhizal association can improve the growth of *G. arborea* in arsenic contaminated soil. Mycorrhizal symbiosis is a key factor which helps plants to cope with the adverse environmental conditions. Beneficial effects of mycorrhiza on plant growth under arsenic contaminated soil have been demonstrated in various other plant species (Sharples et al. 2000; Yun-Sheng et al. 2007; Ultra et al. 2007). Arbuscular mycorrhizal (AM) fungi can improve plant tolerance to toxicity and can uptake toxic substance from soil (Leyval et al. 1997). Plants get benefited from mycorrhizal symbiosis mainly due to the increased absorptive surface provided by fungal hyphae in their root system (Smith and Read 1997). Growth of mycorrhizal and non-mycorrhizal plants, therefore, may respond differentially to soil nutrient concentration, as observed in several experiments (e.g. Bougher et al. 1990; Titus and del Moral 1998).

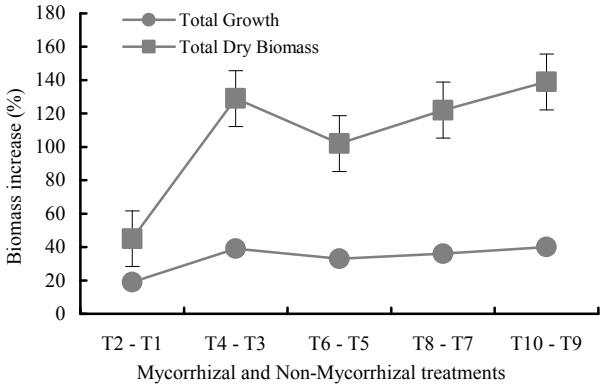


**Fig. 3 Difference (%) between total growth (shoot length + root length) and total dry biomass of mycorrhizal and non-mycorrhizal seedlings of *G. arborea***

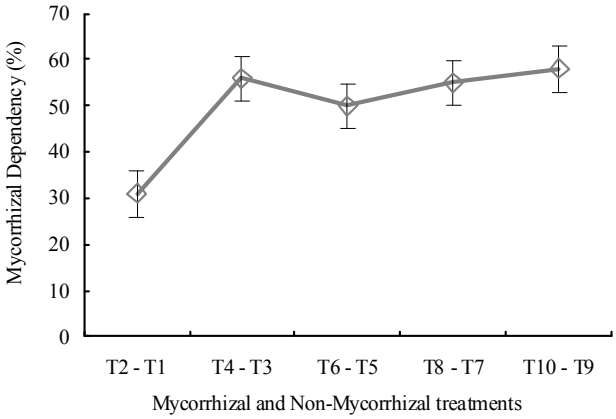


**Fig. 4 Mycorrhizal dependency (MD) of *G. arborea* seedlings**

Mycorrhizal seedlings, in this experiment, had higher shoot height, root length, collar diameter and biomass production. Significant enhancement in root and shoot growth of nursery-raised seedlings may be due to increasing supply of nutrients (Giri et al. 2005), carbohydrate partitioning (Graham et al. 1997) and toxicity mediating ability (Dong et al. 2008) of mycorrhizal fungi. Reduction of both root and shoot length is a typical response to toxic metals (Kabata-Pendias and Penias 1984). Sharples et al. (2000) reported that mycorrhizal fungi can filter arsenic to maintain a tolerable limit in plant body and at the same time provide adequate nutrient to the host as a result of symbiosis. Similar observation of higher growth in mycorrhizal seedlings in arsenic contaminated soil was found by other researchers (Ahmed et al. 2006; Dong et al. 2008). Decrease in number of leaves in plant due to arsenic exposure is an important factor to be considered as it impedes plant physiological activities by reducing net photosynthetic area which directly affects root growth and biomass production (Marin et al. 1993). Reduction in root length due to arsenic exposure has been reported in several studies (Meharg and McNair 1992; Sneller et al. 1999; Hartley-Whitaker et al. 2001). Spreading of plant root is heavily disturbed by the toxic element in soil. High arsenic concentration in soil can stop usual root functionality (sap transportation, nutrient uptake, gas diffusion etc.) and plants are compelled to shorten their root length in toxic environment to stop the association of root cap region to be the first arsenic contact point (Ahmed et al. 2006). Carbonell-Barachina et al. (1997) reported that arsenic causes disruption of root functions, resulting significant reduction of nutrient uptake by plant. AM fungi are well known for its ability to modify root exudation, carbohydrate metabolism and rhizosphere populations of host plant. The mutualistic association between fungal hyphae and plant roots act as a net for arresting soil organic matter and soil minerals. Exudates (e.g. glycoprotein, polysaccharides etc.) from root and fungal hyphae act as glue in adhering soil particles together and give more structural stability. The secreted biomolecules as a result of mycorrhizal symbiosis assist host plants in their growth and strengthen their immune system. Biomolecule produced by fungal hyphae such as glomalin helps host plants to reduce the level of toxicity in soil by converting it into organic form and making it more bioavailable (Sanon et al. 2006; Chern et al. 2007).

Significant increase in total dry biomass of mycorrhizal seedlings was observed in the present study and it was up to 2.4 times higher than that of non-mycorrhizal seedlings. The increased biomass production of mycorrhizal seedlings may be due to better root development, which in turn promoted dry matter weight in the seedlings. Similar findings were also reported by other researchers (Das et al. 1997; Nwoko and Sanginga 1999; Prasad 2000). This indicates that plants' ability to channel energy for shoot production was increased due to the symbiotic association of AM fungi with plant root system (Benthlenfalbay et al. 1982). AM fungi have important role in increasing nutrient absorbing area of plant root through its

extraradical mycelium. The increased mineral nutrients can improve the nutritional condition of plant and thus increase plant productivity (Yun-Sheng et al. 2007). Several other studies (Li et al. 1991; Marschner 1995; Christie et al. 2004; Joner et al. 2000) also demonstrated beneficial effect of mycorrhiza in increasing biomass and P nutrient status of plant in highly metal contaminated soils.

AM fungi protect plants from the toxic effect of non-essential chemical in shoots by retaining these chemicals in their root systems (Ultra et al. 2007). Meharg and Hartley-Whitekar (2002) reported that mycorrhizal plants showed an enhanced resistance to arsenic in highly contaminated soils. The role of AM fungi as a filter of arsenic in plant was also well reported by several other recent and past studies (Asher and Reay 1979; Sharples et al. 2000; Ultra et al. 2007). AM fungi change chemical structure of root exudates and modify the rhizoshpere environment to produce more mycelium which acts as a nutrient source for the microorganisms in plant root region (mycorrhizosphere) (Ultra et al. 2007). Barea et al. (2005) figured out an arsenic mediating mechanism of AM fungi. According to them, symbiotic association of mycorrhiza and plant root enhances indigenous microflora with an arsenic metabolizing ability in rhizoshpere region and trigger arsenic co-metabolism between plant and mycorrhizal fungi. This process also paves the way of biomethylation with a direct involvement of AM fungi. Fungi, bacteria and actinomycetes in rhizosphere zone use this biomethylation process to transform inorganic arsenic to organic form.

Higher mycelium, vesicle and arbuscule infection (%) as well as spore population were observed in root and rhizosphere soil of mycorrhizal seedlings. With increasing arsenic concentration in soils, fungal activity in the rhizosphere region probably was reduced considerably, which stopped spreading of fungal hyphae in plant root systems, thus lowered infection rate. Previous studies found a range of mycorrhizal responses to the presence of toxic metals (e.g. Chao and Wang 1991; Vidal et al. 1996). Ahmed et al. (2006) reported significant reduction and complete inhibition of AM colonization in the root of seedlings planted in metal polluted soils. Turnau et al. 1996 demonstrated that mycorrhizal plant grown on metal contaminated soil (Cd & Zn) had higher AM colonization rate than non-mycorrhizal plants. Mridha and Dhar (2007) found that *G. arborea* seedlings planted in mycorrhiza treated soils had 70% higher root colonization than the seedlings grown in soils without mycorrhiza, although their experiment did not cover arsenic toxicity status. Dong et al. (2008) found higher infection rate in mycorrhizal seedlings planted in soil with lower arsenic concentration. Their findings also indicated that higher mobility of fungal hyphae is needed for higher symbiotic activity and hyphal mobility is stopped in an elevated toxic environment. Ahmed et al. (2006) found that arsenic addition above 1 $mg \cdot L^{-1}$ significantly ($p < 0.001$) reduced the mycorrhizal infection percentage in plant roots. At the highest level (10 $mg \cdot L^{-1}$) of arsenic addition, they observed only 6% of the root length was infected by mycorrhiza. Higher fungal spore population in less arsenic polluted soil can be explained by higher fungal colonization intensity in respective treatments. Probably, soils with less toxicity favored fungal community to produce more spores in rhizosphere zone. Increased availability of mycorrhizal propagules ensures vigorous colonization in plant roots as a result develops an intense hyphal network (Friese and Allen 1991). In an experiment with herbaceous plants, Pawlowska et al. (1996) found 96% colonization intensity and spore populations of 25 (per 100 g of rhizosphere soil) for plants grown in contamination free soil and, 76% root colonization and 20 spores (per 100g of rhizosphere soil) for plants grown in metal contaminated soils. The lesser root colonization and spore population in their study were explained as an effect of toxicity and contamination in soils. Finding of this study seems to corroborate that of Pawlowska et al. (1996).

There was significant positive correlation between total dry biomass of *G. arborea* seedlings and percentage of AM infection (%). Higher infection in the root system can ensure higher availability of nutrients for plants. According to van der Heijden et al. (1998), nutrient exploitation ability of plants results from increasing AM fungal association and hyphal length in root. However, hyphal length measurement was not taken in this experiment. The results suggest that a well-developed mycelial network could increase the biomass in plants by evenly distributing available resources in the soil environment. Mycorrhizal fungi are known to have mediating effect of toxic chemical by modifying micro-organism community in soil. Soil micro-organisms can inactivate or change the chemical make-up of toxic compounds which protect seedlings from harmful effect (Sanon et al 2006).

Mycorrhizal Dependency (MD) is the extent at which a plant species relies on mycorrhizal symbiosis for producing maximum biomass at a given level of soil fertility (Giri et al. 2000). In our experiment, mycorrhizal dependency of *G. arborea* seedlings was variable among different treatments. Highest dependency (%) was found in treatment with highest arsenic level ($T_{10}$; 100 $mg \cdot kg^{-1}$). This phenomenon can be explained as a reflex action of affected plants in maintaining their physiological activities in a high toxic environment. Strong symbiosis and mycorrhizal dependency of plants grown on high level of arsenic-contaminated soil in the current experiment explained the role of mycorrhiza in buffering contamination effect of arsenic. Giri et al. (2005) and Dong et al. (2008) also found higher mycorrhizal dependency of plant grown in arsenic contaminated soil. In the current study, comparatively lower MD was observed in treatment $T_6$ despite higher level of arsenic concentration in that treatment. This might be due to the difference in AM fungal species or age of the inoculums in specific treatment happened by chance. However, the increasing trend of mycorrhizal dependency of seedlings with increasing concentration of arsenic in soil is evident from the current results.

*Leucas aspera* root was used as natural mycorrhizal inoculums in the current experiment. This species is studied well and found to have good mycorrhizal association (100% infection found in this experiment) in their root system

(Muthukumar et al. 2006). It is a widely grown species in Bangladesh and adapted with local climate (Sadhu et al. 2003) which also increases the inoculation potential of mycorrhiza in known soil environment. Plantation cost can substantially be reduced as well by using this locally available inoculum instead of buying commercially available mycorrhizal spores.

The arsenic problem is now appeared as a threat for biosphere. The risk of arsenic contamination in tree species has received little attention until now. From this observation, it was clear that mycotrophic plants were less affected in arsenic contaminated soil than the non-mycotrophic plants. Our experiment revealed that different concentrations of arsenic have significant difference in the level of toxicity on the growth of *G. arborea*. With increasing arsenic concentration, plant growth, biomass and mycorrhizal colonization intensity decreased significantly. Inoculation of mycorrhiza in arsenic contaminated soil has improved the growing conditions of plants, significantly. Mycorrhizal seedlings showed better performance in terms of growth, biomass and fungal colonization than non-mycorrhizal seedlings. Although, arsenic uptake and chemical transformation by seedlings were not tested in this study, these findings gave an indication that use of effective AM inoculants may help the transformation of toxic arsenic into less toxic form. This can be used as a promising technology for mediating the harmful effect of arsenic on growth of *G. arborea* at nursery stage. Further research is still needed to reveal the detail mechanisms of arsenic transport and translocation in plant parts, finding threshold limit of arsenic resistance in plants as well as field level performance of this technique.

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